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TUBERCULIN TESTING OF LIVESTOCK

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IMPORTANCE OF TUBERCULOSIS ERADICATION

Tuberculosis of the food-producing animals is probably the most serious disease with which the American farmers are confronted. Realization of this fact resulted in the organization of the cooperative campaign for its control and eventual eradication between the United States Bureau of Animal Industry, the State livestock sanitary officials, the livestock owners of the Nation, and other persons interested in the livestock industry. The disease has been regarded with such apprehension as to lead to a more detailed study of the question of control and eradication, especially by members of the veterinary profession. If tuberculosis is to be controlled or eradicated, it is necessary to have a clear conception of the peculiarities of the disease, its nature, its mode of dissemination, and, after this, a wide experience in the use of the various methods of detection. The many important phases of the tuberculosis problem mentioned have been the subject of articles and discussion by scientists since Robert Koch discovered the active cause of the disease and later prepared the biological product known as tuberculin. In the literature and discussions for many years much thought has been given to the methods advocated for the detection of the disease. These methods are chiefly interesting to the veterinarian who desires definite knowledge of those which are recommended and which have proved to be successful.

Various methods of diagnosing tuberculosis in the living animal have been advanced, such as physical examination, use of the sputum cup, laboratory examination of milk, feces, and other product, and the use of tuberculin. This circular deals only with the various tuberculin-test methods. The other methods have their value when

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used in conjunction with tuberculin testing, but can not be relied upon alone to detect all tuberculous animals in infected herds. In presenting data relative to the various methods of testing, the experience gained by veterinary inspectors engaged daily in tuberculin testing is the best index for the substantiation of the instructions and suggestions here made. The methods advocated and results reported have met with the approval of livestock owners who have placed their herds under the joint supervision of the Bureau of Animal Industry and State forces. In this cooperative work from July 1, 1917, to December 1, 1927, approximately 43,500,000 tests have been made, which has afforded an excellent opportunity for a careful study of the various tests.

NATURE OF TUBERCULIN

Tuberculin is a product prepared by sterilizing, filtering, and concentrating the liquid upon which tubercle bacilli have been allowed to vegetate. It contains the cooked product of the growth of the bacilli, but not the bacilli themselves, and is used in several forms for the diagnosis of tuberculosis in animals. It was first made and studied by the great scientist, Robert Koch, who found in 1890 that if he injected it into the tissues of a tuberculous animal it had the effect of causing a decided rise of temperature, while it had no such effect upon animals free from tuberculosis.

This product was tested by many investigators during 1890 and 1891. It was recognized at that time as a most remarkably accurate method of detecting the disease even in the early stages when it had made but little progress, and it is interesting to note that the old tuberculin (O. T.) is still the basis of the product used at the present time. As it is prepared by the Bureau of Animal Industry, 4 cubic centimeters contain 0.5 gram O. T., which is the minimum dose for the subcutaneous test of adult cattle. In determining dosage veterinarians should be guided by the quantity of O. T. in the product they are using. It is also essential to determine the source of the manufacture of tuberculin. Without this knowledge the testing of cattle would become a very inaccurate procedure. All commercial concerns doing an interstate business are under license of the United States Department of Agriculture, and all official State laboratories are supervised by competent officials. Therefore purchases should be made from sources known to produce a potent product.

The present knowledge of tuberculin and its reliability has been built up through careful and thorough scientific experimental work. Its use by any individual who has not been trained to appreciate its value and recognize its manifestations should be discouraged, as in many cases its effect on tuberculous animals would pass unobserved or would not be understood properly. In the hands of unscrupulous persons it is a danger to the livestock industry of the Nation because it is known that some animals may be so frequently treated with tuberculin as to establish a tolerance against its action, or, in other words, they lose their sensitiveness to its action. It should be allowed only in the hands of trained and qualified veterinarians.

QUALIFICATIONS OF THE VETERINARIAN

In studying the history of tuberculosis and the use of tuberculin, the conclusion is drawn that in the past tuberculin testing was regarded by many as a routine operation which required little skill in its application or interpretation of the results obtained. This idea is a fallacy and is not to be entertained. Tuberculin is a very reliable agent and in the hands of the experienced veterinarian will give accurate results. One should remember that in order to obtain the best results a close study of the action of tuberculin is necessary, and in addition the necessary instruments for the proper conduct of the tests should be available. From observation of the work of many men it is deemed necessary to emphasize the close relationship between reliable testing and the accurate conscientious operator. So minute should be the study of the work in hand as to lead to not only a study of the herd under test but also of each individual animal in the herd. Only by such attention to details will the desired results be accomplished. Then, too, the standpoint of the livestock owner must not be lost sight of. He is naturally the most interested party to the transaction and must of necessity, if the work is to be successful, be given such service as fully to convince him of the wisdom of the problem which he has undertaken. A satisfied livestock owner becomes the center of favorable local sentiment toward tuberculosis eradication. It is obvious that sound judgment and diplomacy on the part of the veterinarian are essential to success in testing animals with tuberculin.

OFFICIAL METHODS OF TUBERCULIN TESTING

The several methods of tuberculin testing recognized for use by the Bureau of Animal Industry and various State officials, either alone or in combination, are:

1. The subcutaneous or "thermal" test.
2. The intradermic or "skin" test.
3. The ophthalmic or "eye" test.

A form of the intradermic test known as the intrapalpebral test is that in which the injection is made into the skin of the lower eyelid.

Another common practice among operators is the injection by the intradermic method into the junction of the skin and mucous membrane of one of the lips of the vulva. This test is not used alone but is used in conjunction with an injection made into the caudal fold. This double injection is frequently termed the "upper" and "lower" injection. It has been found to be very effective in helping to arrive at a decision in doubtful cases.

The numbers of tests officially made by the various methods during the period from October 1, 1920, to June 30, 1927, were: Subcutaneous, 568,713; intradermic, 30,227,090; and ophthalmic, 10,517. It is seen, therefore, that all methods have been used to the extent of thousands of tests. Accurate records of the work are kept by bureau officials, and close study has been given to the results obtained.

In discussing each of the methods used and the combinations thereof no comparison will be drawn regarding the relative value or accuracy of any. As has been stated, each method and each combination has certain advantages, and there are times when one should be used

in preference to another. Used under the proper conditions, any tuberculin test properly applied is remarkably accurate. Also, in connection with the discussion of each test, the writers desire to emphasize that the ordinary results obtained in the testing of each animal are either a positive or a negative diagnosis. The experienced operator will have no trouble in making such a diagnosis. It is only in a small percentage of cases where a state of doubt will exist and it is these cases which are being emphasized. Where badly infected herds are encountered, the doubtful case should be classified as a diseased animal. On the other hand, in those herds believed to be free from disease, it is better judgment to class all doubtful cases as suspicious.

THE SUBCUTANEOUS TEST

The subcutaneous method of testing consists in the injection of a given dose of tuberculin under the skin of the animal. Most operators prefer the skin above the point of the shoulder as the seat of injection; however, any convenient point may be selected. This test until recently was the most commonly used for all purposes, and by reason of the fact is possibly better understood by most veterinarians. Until March 1, 1920, it was the only test officially recognized by the Bureau of Animal Industry. The history of its application for many years indicates that in the hands of competent individuals it is very reliable. More recent study and the use of other methods have shown that it probably lends itself more to the assistance of those who desire to defeat its ends than does any one of the other tests. It is probably most accurate when used on animals for the first time. Dr. D. E. Salmon, former chief of the Bureau of Animal Industry, stated in 1906:

> The first test of a herd with tuberculin, combined with the physical examination of the individual animals may be accepted as reliable in its indications not only as to the existence of tuberculosis in the herd but also as to the healthfulness of the various animals composing the herd.

A different aspect is presented in testing animals which have been frequently tested and which have established a tolerance to tuberculin. Under these conditions many of the so-called failures of this test have become apparent. The writers believe that many of these failures of diagnosis can be attributed to the operators' failure to recognize slight reactions or in expecting to find what is termed a characteristic or marked reaction. The apparent failures of the test may be classified under two headings: (1) Those not disclosing infected animals, which results in leaving diseased cattle in the herd: (2) the classifying of animals as reactors when a slight thermal disturbance may be due to other causes. This results in healthy animals being classified as diseased. The latter class is the less important. It is those animals which are diseased and which are left in the herd that cause a continuing infection. In using the subcutaneous test on cattle which have been apparently immunized against the action of tuberculin it should be used only with another method in combination, and larger doses of tuberculin should be employed.

In applying the subcutaneous test it is very essential that veterinarians make careful observations to note the possible physical reaction which sometimes becomes apparent in addition to the thermal

reaction, or which may occur when no temperature rise is indicated. Physical disturbances, however, are rather infrequent in diseased cattle which have received several injections of tuberculin. This physical reaction is usually manifested by one or more of the following symptoms: Depression, trembling, bloat, chill, loss of appetite, suspended rumination, difficult breathing, diarrhea, cold extremities, thirstiness, soreness at the point of injection, and others.

No small part of a successful subcutaneous test lies in the proper preparatory steps. If they are taken, the veterinarian will find the work much lightened and much more satisfactorily carried out. The owner should be advised a sufficient length of time ahead of the expected arrival at the farm to have the animal properly restrained for a period of at least one or two hours before the test is begun. Definite instructions should be given the owners as to what is expected and required of them. This is essential whether the test is an official cooperative one or is a so-called private test. The information should include a statement concerning the assistance required of the owner during the test, some understanding of the method or methods to be used, a statement as to what disposition must be made of reactors, if found, in accordance with the State's regulations, and other instructions relative to sanitation on the premises. The veterinarian should provide himself with clean overalls; he should have the necessary disinfectant for use, well-kept and well-cleaned syringes and needles, and test charts sufficient to record all the animals to be tested. For the average herd of from 20 to 30 head no fewer than one dozen thermometers should be available.

Every veterinarian in applying a test should be equipped with tags to identify cattle which successfully pass the test as well as any reactors that may be found. Immediately before or after taking the first preliminary temperature the identity of the animal should be established upon the test records in the order in which temperatures are taken. Temperatures should be recorded immediately upon being read. Any animal showing an abnormal temperature at any time during the test should be immediately checked by the use of another thermometer. No animal showing an abnormal preliminary temperature should be injected with tuberculin. Occasionally animals register high upon the first preliminary temperature and resume the normal prior to injection. Cases of this character may be accounted for by the animal's having been recently put under restraint and in such cases may be safely injected. Extreme care should be exercised and the fact determined that no pathological condition exists which would cause the elevation of temperature.

Although the ordinary result of a tuberculin test by the subcutaneous method is plainly either positive or negative, interpretations are occasionally difficult to make. Tuberculin reactions vary considerably and a large number of tuberculous animals show no clear, well-defined temperature curve. This is especially true of old cows that have long-standing advanced cases of tuberculosis. Frequently in badly infected herds animals should be classified as reactors which have not reached the maximum of temperature required in a strict interpretation of the regulations. On the other hand in herds believed to be free from the disease an occasional animal will give a sufficient rise in temperature to come within the classification of a

reactor. In accord with experiences which have conclusively proved that "once a reactor always tuberculous" no attempt should be made

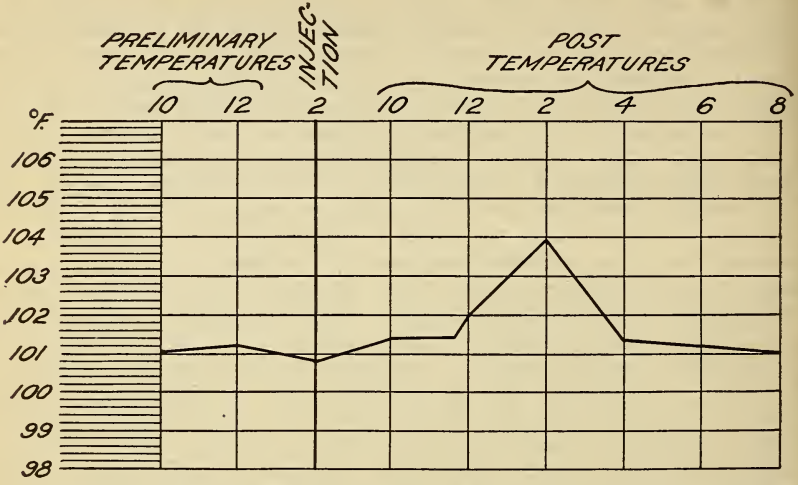

FIG. 1.—Subcutaneous tuberculin-test chart showing sudden rise and fall of temperature between second and fourth post temperatures. This animal should normally be classified as suspicious unless accompanied by a physical reaction or other evidence of infection, in which case it should be classed as a reactor

to find excuses for a typical reaction. However, judgment must be used in classing animals as reactors under conditions in which a reasonable doubt exists as to their condition. This judgment is

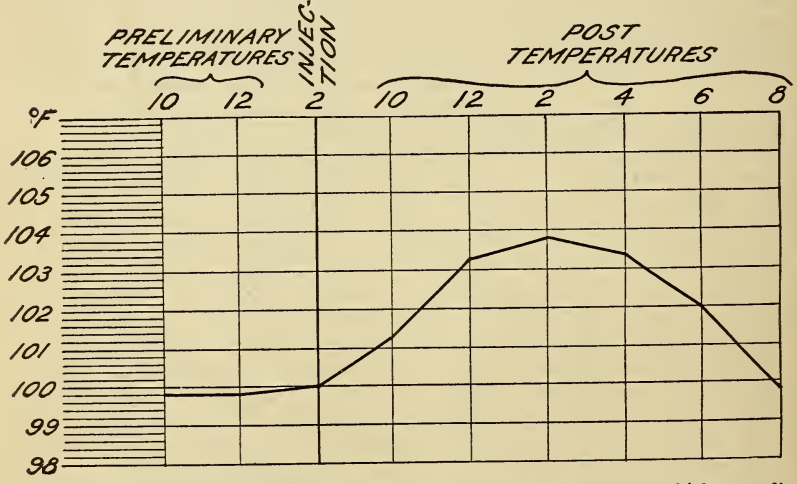

FIG. 2.—A typical subcutaneous-test curve with a maximum temperature which according to a strict interpretation of instructions would necessitate classifying an animal as suspicious. However, if the animal is aged and in poor condition and in a tuberculous herd, it should under those conditions be classified as a reactor. (Note that temperatures are not so high as in fig. 3)

acquired as the result of proper training and practical experience. Table 1 and Figures 1 to 6 show the different types of reactions and how they may be classified under varying conditions.

TABLE 1.—*Different types of reactions to the subcutaneous tuberculin test and how they may be classified under varying conditions*

No. of animal	Description	Condition	Age	Sex	Weight	Preliminary temperatures, July 28, 1921			Amount injected	Time of injection	Postinjection temperatures, July 29, 1921						Disposition
						4 p. m.	6 p. m.	8 p. m.			4 a. m.	6 a. m.	8 a. m.	10 a. m.	12 m.	2 p. m.	
			Years		*Pounds*	°*F.*	°*F.*	°*F.*	*C. c.*		°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	°*F.*	
1	Black and white	Fair	5	F	900	101.0	101.2	100.8	5	8 p.m.	102	102	103.8	101.4	101.2	101	Suspect.
2	Roan	Poor	10	F	850	99.8	99.8	100	8	8 p.m.	101.2	103.4	103.8	103.4	102	99.8	Reactor.
3	Red	Fair	2	M	650	102.2	102	102	4	8 p.m.	102	102	103.8	103.6	102.8	102	Suspect.
4	White	Good	6	F	1,000	101.8	102.2	102	4	8 p.m.	101	102	104	104.8	105.6	105	Reactor.
5	Red and white	do	5	F	750	101.2	102.2	102	4	8 p.m.	105	106.2	104.2	104	105	104	Do.
6	Jersey	Poor	10	F	700	100	101.2	101	8	8 p.m.	100.2	101.8	101.4	103.8	103.6	103.6	Do.
7	Roan	Good	2	M	1,100	102.4	102.6	102.4	4	8 p.m.	102.6	102.4	103	102.8	103.2	102.4	Healthy.

NOTES

No. 1.—Should normally be classified as a suspect unless accompanied by a physical reaction or other evidence of disease, in which case it should be classed as a reactor.

No. 2.—Under a strict interpretation of the instructions this animal would probably be classified as a suspect. Taking into consideration the poor condition of the animal, its age, and the fact that it was contained in a lot of diseased cattle it should be classified as a reactor.

No. 3.—Under a strict interpretation of the instructions this animal should be classified as a suspect. If contained in a lot of diseased cattle and if any indication of a physical reaction is apparent, it should be classified as a reactor.

No. 4.—Is a typical reactor, and should under all conditions be classified as such.

No. 5.—This animal does not show a so-called "rainbow curve" but without question should be classed as a reactor. This does show the so-called "plateau."

No. 6.—The same conditions exist here as indicated for No. 2.

No. 7.—This animal, young in age and fat and evidencing a maximum postinjection temperature of 103.2° F., should be classified as healthy.

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ADVANTAGES AND DISADVANTAGES

The advantages and disadvantages of the subcutaneous method of testing are as follows:

Advantages.—It entails more detailed information upon the test records, necessitating the constant attention of the veterinarian and resulting in a very close study of the herd and animals under test. It lends itself readily to combination with the ophthalmic method. It is usually very accurate when applied to an animal or herd for the first time.

Disadvantages.—Erratic temperatures make it inadvisable to use this test upon very young animals or animals which are hard to restrain. The constant attention which it necessitates makes for physical hardship upon the veterinarian. It is slower than other methods, limiting the testing to a smaller number of cattle at one time, with consequent increase in the cost.

DIRECTIONS FOR APPLYING

The following gives in detail the technic of the subcutaneous method of testing:

1. So far as practicable the cattle should be stabled under usual conditions and with usual surroundings.

2. The cattle should be fed and watered in the customary manner, except that it should be done immediately after the taking of temperatures. Occasionally it is advisable to limit the quantity of concentrated feed or hay given animals under test. This is especially true if large quantities of feed are allowed.

3. A careful physical examination of each animal should be made before or during the application of the test.

4. During the period immediately before the injection of tuberculin each animal's temperature should be taken at least three times at not less than two-hour intervals. Care should be taken to let the thermometer remain inserted long enough to insure accurate registration.

5. Animals showing evidence of any acute disease or condition or showing pus formation or pronounced abnormal preliminary temperature should not be injected with tuberculin. Inquiry concerning the history of the herd should be made, and it should be ascertained, if possible, whether any animal in the herd has ever given a positive reaction to tuberculin, the number of tuberculin tests applied to the herd previously, and also whether any cattle in the herd have been treated at any time in any other manner with tuberculin. Any reactor to a previous test should not be retested. The information thus obtained should be used in determining the method to be employed in the tuberculin testing of the herd.

6. The hypodermic syringes and needles should be disinfected before treating any herd with tuberculin. Before being used for the injection of each animal needles should be washed in a disinfectant solution. A 5 per cent solution of carbolic acid is suitable for sterilizing the instruments. Open bottles of tuberculin should be protected from contamination.

7. Thermometers should be sterilized before the temperatures are taken and should be dipped in a disinfectant solution before and after each reading.

8. For cattle which are apparently healthy and have not been injected with tuberculin within a period of at least 60 days the dose of tuberculin prepared by the Bureau of Animal Industry is as follows:

2 cubic centimeters (0.25 gram O. T.) for calves up to 1 year of age.
4 cubic centimeters (0.5 gram O. T.) for cattle more than 1 year old and up to maturity.

Older animals, repeatedly tested animals, or those clinically suspicious may receive a larger dose. The dosage administered should be based mainly on the ages of the animals and on their history rather than on their weight.

9. The taking of temperatures following the injection of tuberculin should commence not later than the eighth hour and be continued every two hours until the eighteenth hour after injection when, if there is no tendency for the temperature to rise, the test may cease.

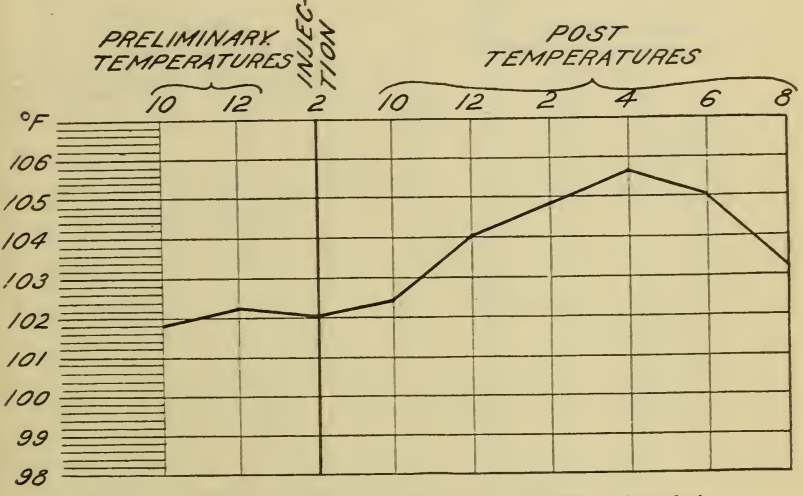

FIG. 3.—A so-called typical "rainbow" curve, indicating tuberculosis

Temperatures of cattle which show a rising tendency following the injection of tuberculin should be taken more frequently and over a longer period of time in order to insure an accurate diagnosis.

10. Experience has shown that animals, especially those of doubtful record, receiving large doses of tuberculin, may respond early to the test, and inspectors are advised to obtain temperatures wherever practicable at from the fourth to the sixth hour on this class of cattle.

11. Suspected cattle should be submitted to a retreat after 60 days. This class of cattle and those which show possible physical evidence of tuberculosis, emaciation, old age, or which have been tested repeatedly should receive double the dose of tuberculin indicated above, and should also be check-tested by the ophthalmic method.

12. A rise of 2° F. or more above the maximum temperature observed prior to the injection of tuberculin or a temperature above 103.8° F. should be regarded as an indication of tuberculosis provided the temperature reaction shows the characteristic "rainbow" curve. (See fig. 3.) An elevation of temperature higher than

103.8° F. should be also regarded as an indication of tuberculosis even though the so-called rainbow curve is replaced by what is termed "the plateau." (See fig. 4.)

13. Animals which after injection show a rise in temperature of 2° F. with a maximum of between 103 and 103.8° F., as well as those which show a rise of less than 2° F. with a maximum temperature of 103.8° F., are regarded as suspicious. The presence of a general systemic reaction or a typical curve should be considered a determining the classification between suspects and reactors.

THE INTRADERMIC TEST

The intradermic method of testing consists in the injection of tuberculin between the layers of the skin. Various points may be

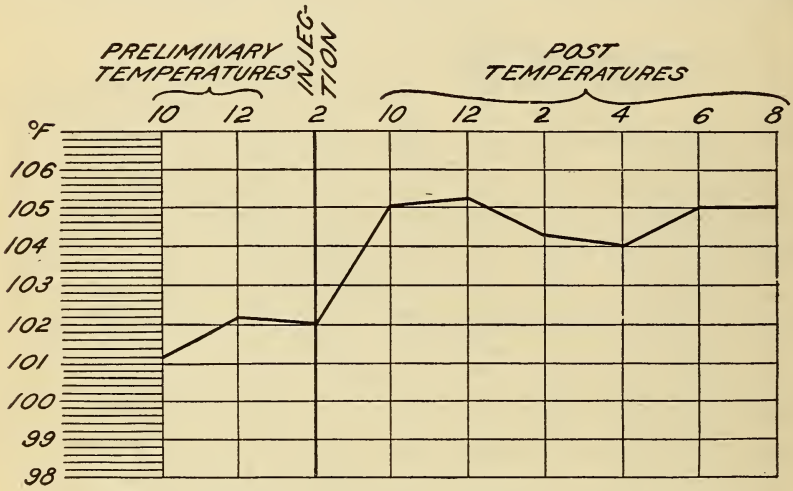

FIG. 4.—Type of temperature curve called the "plateau," indicating tuberculosis

selected for injection. Most operators, however, prefer to inject the tuberculin into the skin of the caudal fold on either side of the tail at a point two-thirds of the distance from the base. When the injection into the vulva is made it is usually in the lower third of either lip at the junction of the hair and mucous membrane. The writers believe that at these points the absorption is easily accomplished and that observations are relatively easy because of the comparison offered by the uninjected, opposite caudal fold or lip of vulva. Experimental work was conducted by State and bureau employees prior to March 1, 1920, on which date the intradermic method was recognized as official by the Bureau of Animal Industry. It has been used for a number of years in California, Missouri, Montana, Texas, Wisconsin, and other Western States. As has been stated, between October 1, 1920, and June 30, 1927, 30,227,090 tests were made by this method under the official cooperative tuberculosis-eradication plan, with good results.

This work has proved this method of test equally as reliable as any other method and it has certain advantages not possessed by

the subcutaneous or ophthalmic methods. It lends itself peculiarly to circumscribed-area eradication work, permitting the testing of a larger number of cattle that would otherwise remain untested, possibly for years, thus checking the automatic increase in the percentage of infection. The work already conducted on the area basis in various States is convincing proof of the excellent results following the proper use of the intradermic test, notwithstanding some criticism based on inadequate knowledge of technic and test records. If the proper technic is employed, no more uncertainty will exist when the observations are made than is occasionally found when

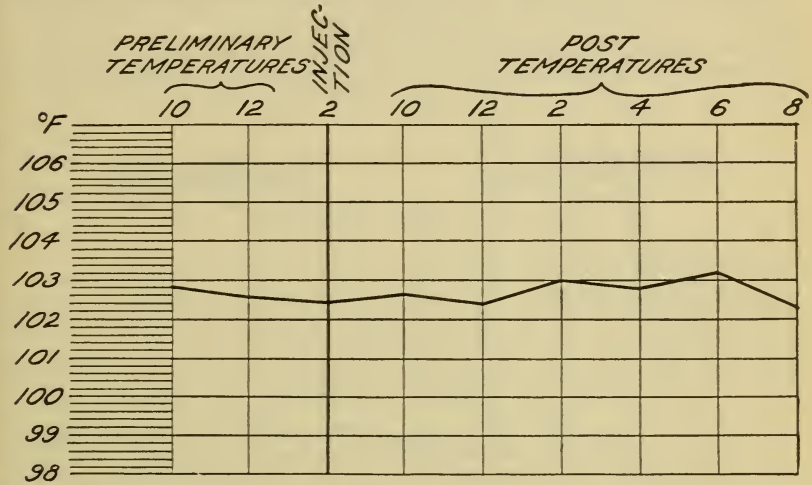

FIG. 5.—Temperature curve showing a maximum of only 0.4° F. between the highest preinjection and postinjection temperatures. An animal showing such a temperature should normally be classified as healthy

other tests are used. Figure 7 shows a very pronounced intradermic reaction.

ADVANTAGES AND DISADVANTAGES

The advantages and disadvantages of the intradermic test are as follows:

Advantages.—It is equally as reliable as any other test applied alone. It facilitates the work, the operator being able to test a much larger number of cattle in a given time. Animals of all ages, dispositions, and those maintained under adverse conditions can be tested without confusing the operator. It lends itself readily to combination with the ophthalmic test. It pleases the livestock owner, especially the dairyman, who is not required to confine his cattle for a very long time.

Disadvantages.—Its apparent simplicity may induce carelessness on the part of a veterinarian not thoroughly trained and experienced. The injection is a delicate operation and unless the cattle are properly restrained it is not very easily accomplished.

The question frequently arises as to the possible interference of a previous subcutaneous test with the intradermic test, or a succeeding subcutaneous test following an intradermic injection. It is believed that there is a possible influence of one upon the other and it

is, therefore, desirable to permit as long a period of time as possible to elapse between the injections of tuberculin by any of the methods except the ophthalmic. This, of course, does not apply when the so-called triple combination test is used, a subject that will be discussed further under the head of Combination Tuberculin Tests.

A form of intradermic testing known as the intrapalpebral test consists in the injection of a minimum dose of tuberculin into the layers of the skin of the lower eyelid. The results obtained from the proper administration of tuberculin by this method are very good. Because of the very delicate nature of the operation, however, and the difficulty in properly restraining animals for the intrapalpebral test, it is not recommended for general use.

There are differences of opinion regarding certain principles of the intradermic test and some lack of uniformity exists as to methods of

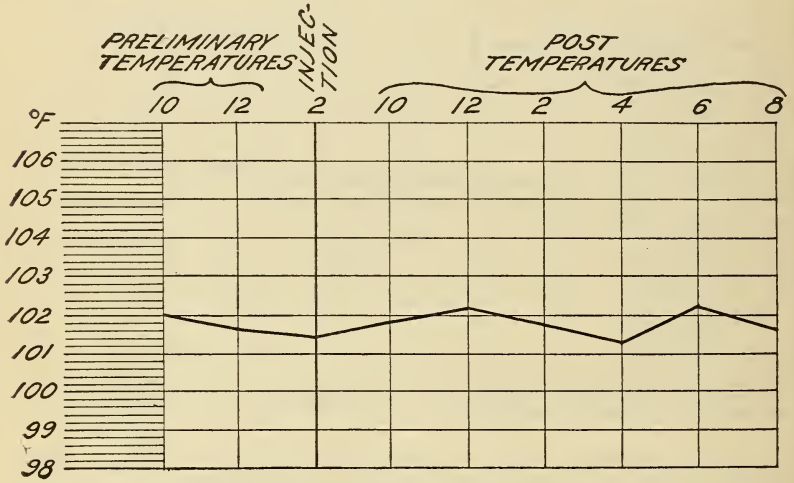

FIG. 6.—Curve showing the temperature range of the average healthy animal

injection and diagnosis. In connection with the type of syringe and needles recommended for use under the detailed technic for the test it is desirable to specify briefly why such types of instruments are deemed preferable. The syringe recommended is of a comparatively long, narrow-barrel type which permits an easy use of a long, straight needle. It further aids in determining when a given dose has been injected by the ability of the operator to feel the pressure on the plunger, which is not possible with a syringe of a larger type. The objection that such a syringe requires constant refilling should not be considered as of any importance. A needle with a full half-inch extension is deemed advisable unless wild cattle are to be injected, because, among other reasons, of the additional care required of the operator in using one of this type. When a short needle of a larger gauge is used there may be a tendency upon the part of some operators to become careless in making an injection. With a long needle such procedure requires a restraint of the animal and a very careful handling of the tissues to avoid making too deep or too superficial an injection. In addition to these reasons it is believed that a

needle of this length, which being drawn back approximately half of its length after being fully inserted and before the injection is completed, establishes a track for the dose of tuberculin without unnecessarily breaking down the connective tissue between the layers of the skin. This has been proved to reduce by approximately 95 per cent the occurrence of small shotlike swellings which are not diagnostic of tuberculosis. The veterinarian will be materially assisted if he observes the following principles in applying the test:

Proper equipment should be available.
Animals should be properly restrained at the time of injection.
The seat of injection should be properly cleansed without the use of irritating disinfectants.
A graduated dose of tuberculin should be used.
Injections should be made uniformly at approximately the same point in either caudal fold.
A record of all injections should be taken at the time they are made, and special care taken to indicate those which may not be regarded as entirely satisfactory.
Definite periods for observations must be prescribed and a minimum and maximum time observed.

DIRECTIONS FOR APPLYING

1. The operator should provide himself with the following equipment: (1) A roll of medicated cotton; (2) a suitable syringe, preferably a 25 to 40 minimum small-barrel instrument, which should be cleaned and disinfected between tests; (3) the needles should be of the Schimmel type, 25-gauge and preferably half-inch extension. A shorter needle is advisable in testing animals which are very hard to restrain, such as those of the range type or those which are injected while passing through cattle chutes; (4) after injecting each animal needles should be dipped in medicated or denatured alcohol or wiped with a piece of cotton saturated in alcohol; (5) a container which permits the immersion of the syringe and needles while going from herd to herd; (6) a standard bull lead, with handles, which should be dipped in a disinfectant each time it is inserted in an animal's nose.

2. Cattle should be properly stabled in order that the injection may be facilitated.

3. An assistant should properly restrain the animal, if necessary, by the use of a nose lead or by other methods. It is of prime importance to have the animal so that it will not move upon the insertion of the needle.

4. The seat of injection should be cleaned with alcohol or other cleansing fluid. Disinfectants are not recommended, as they may irritate sensitive skins and cause confusion when observations are made.

5. Examine both caudal folds and note on test record any abnormalities or peculiarities.

6. The animal having been restrained and the seat of injection cleaned, the veterinarian by the most convenient method inserts the needle between the layers of the skin, care being taken to inject neither too deeply nor too superficially. If a half-inch needle is used it is practicable at any time while inserting the needle to verify the accuracy of the injection by slightly lifting the needle, the outline of which will appear under the skin at the point at which the

injection is made. Having inserted the full length of the needle it is carefully drawn back approximately one-fourth of an inch and the tuberculin inserted in the track thus established by the needle. Having injected the tuberculin the needle is carefully withdrawn and, if necessary, the finger and thumb of either hand held lightly on the point of injection to prevent the possible escape of any part of the dose. This method of injection causes the least possible disturbance to the tissues involved. The injection is a very important part of the test and should always be done in a careful manner. The injection into the vulva should be made in an equally careful manner.

7. The dose of tuberculin recommended should not exceed 2 minims of specially prepared intradermic tuberculin, and should be regulated by the gauge on the syringe.

8. The first observation should be made on or about the seventy-second hour after the injection. If the infection of tuberculosis is in the herd, a second observation should be made on or about the one hundred and twentieth hour after the injection and, if it appears necessary, an observation should be made again on or about the one hundred and forty-fourth hour.

9. Animals should be classified as reactors to the intradermic tuberculin test which show, at the point of injection, swellings that may be either hard and circumscribed or soft and infiltrated with no distinct line of demarcation. Such swellings may be of various sizes, from those hardly perceptible to the naked eye to those as large as a human fist or larger. (See fig. 7.) In herds showing infection very small infiltrations or enlargements, other than the shotlike nodules sometimes found at the point of injection, should be classed as reactions. Sound judgment, however, should be used in classifying animals, the previous history of the herd relative to infection being taken into consideration in classifying very small swellings. The ability to make these distinctions is by some called intuition. Without doubt it is efficiency resulting from proper professional training coupled with long practical experience.

10. Records of complete tests should be made upon prescribed forms, and the observations should be reported in accordance with the following code which was adopted by the United States Live Stock Sanitary Association, and approved by the Bureau of Animal Industry on December 6, 1920.

THE INTRADERMIC-TEST CODE

Animals showing no reaction shall be recorded at each observation as "N" (negative).

Reactors shall be recorded as follows:

For circumscribed swellings, "pea" size (diameter $\frac{3}{16}$-inch) shall be used as the basic standard. Larger swellings shall be reported as P2, P3, P4, P5, etc., the figures 2, 3, 4, and 5 referring to twice, thrice, four times, and five times the size of a pea.

For diffused swellings "thick 2 X" shall be used as the basic standard and signifies a diffuse swelling in which the injected caudal fold is twice as thick as the normal fold. Larger swellings shall be recorded as "thick 3 X," "thick 4 X," etc.

THE OPHTHALMIC TEST

The ophthalmic test is made by treating the eye with a specially prepared liquid tuberculin or by the use of tuberculin disks. The disks are regarded as preferable for use because of the comparative ease with which they are applied and the uniformity of the dosage contained in them. This test was used experimentally in a number of the States and by the Bureau of Animal Industry for several

FIG. 7.—A very pronounced reaction to the intradermic test. All swellings resulting from the injection of tuberculin by this method are not so large as indicated in the picture

years prior to December 3, 1920, on which date it was officially recognized as a test to be used in combination with other methods. Much information was obtained indicating its value as a check test. A very limited amount of work has been conducted in applying it alone. This has not been sufficient to give it the unlimited recognition which has been granted the other methods of test. It succeeds frequently in assisting to identify reactors in badly infected herds which would be difficult for the operator to recognize as diseased animals if only one method were used. The method of placing the tablets originally consisted in placing them under the lower eyelid, near the inner canthus. This, while at times satisfactory, did not produce uniform results. It was not until the practice of placing them under the upper eyelid near the external

lateral angle was adopted that particularly uniform results were obtained. This practice is recommended for general use either in applying the disks or the liquid form of ophthalmic tuberculin.

Details of the installation of the disks are given under the instructions for the application of the test. Several precautions need to be used in its application. Cattle can not be safely tested by this method if they are kept in open barns or corrals where sand and dirt can blow freely through, nor if they have been shipped to public stockyards, in which cases their eyes are frequently affected by cinders or infected material. In certain Western and Southwestern States cattle on the open range are frequently annoyed by burs which sometimes lodge in their eyes, causing severe disturbances. A reaction to the ophthalmic test is not to be disregarded merely because it does not check with the findings of the subcutaneous or intradermic methods applied in combination. When the test is used under proper conditions, a reactor to the ophthalmic method can be as safely regarded tuberculous as though it reacted to any other. Figure 8 shows a very typical reaction to this method of test. Emphasis is laid upon the necessity of taking frequent and continuous observations to make a proper diagnosis.

ADVANTAGES AND DISADVANTAGES

The chief advantages of the ophthalmic test are: Very frequently the action of ophthalmic tuberculin seems to be accentuated by previous injections given subcutaneously; the animal that once becomes sensitive to the disks will usually react on repeated treatments. It seems to be especially valuable in those cases in which the disease has progressed to an advanced stage. It lends itself readily to combination with either of the other methods. Its action is in most cases apparent to the livestock owner, who is thereby satisfied with the test.

The principal disadvantage is that the tuberculin is used on a sensitive organ which by reason of injury may show results not always clear to the operator, who may not make a searching examination of the eye.

DIRECTIONS FOR APPLYING

1. Cattle should be properly stabled, with a sufficient number of assistants to restrain the animals while the disks are being inserted.

2. Cleanse the hands before beginning the test and see that the finger nails are pared sufficiently not to injure the eye of the animal.

3. Examine both eyes of the animals to be injected, and be sure not to treat any abnormal eyes or a herd in which sore eyes are prevalent.

4. The test produces the best results when a sensitizing disk is used prior to the installation of the diagnosis disks.

5. The disks are best inserted in the following manner: For the purpose of uniformity the left eye should invariably be the treated eye. An attendant should hold the head of the animal. The operator, standing in front, gently raises the upper lid of the left eye with the left forefinger and thumb. With the right forefinger and thumb he places a single disk under the upper lid at the external lateral angle. The disk being placed the finger and thumb are gently withdrawn

and the left hand held lightly over the eyelid without massaging for about half a minute in order that the disk may not be displaced by the winking eye.

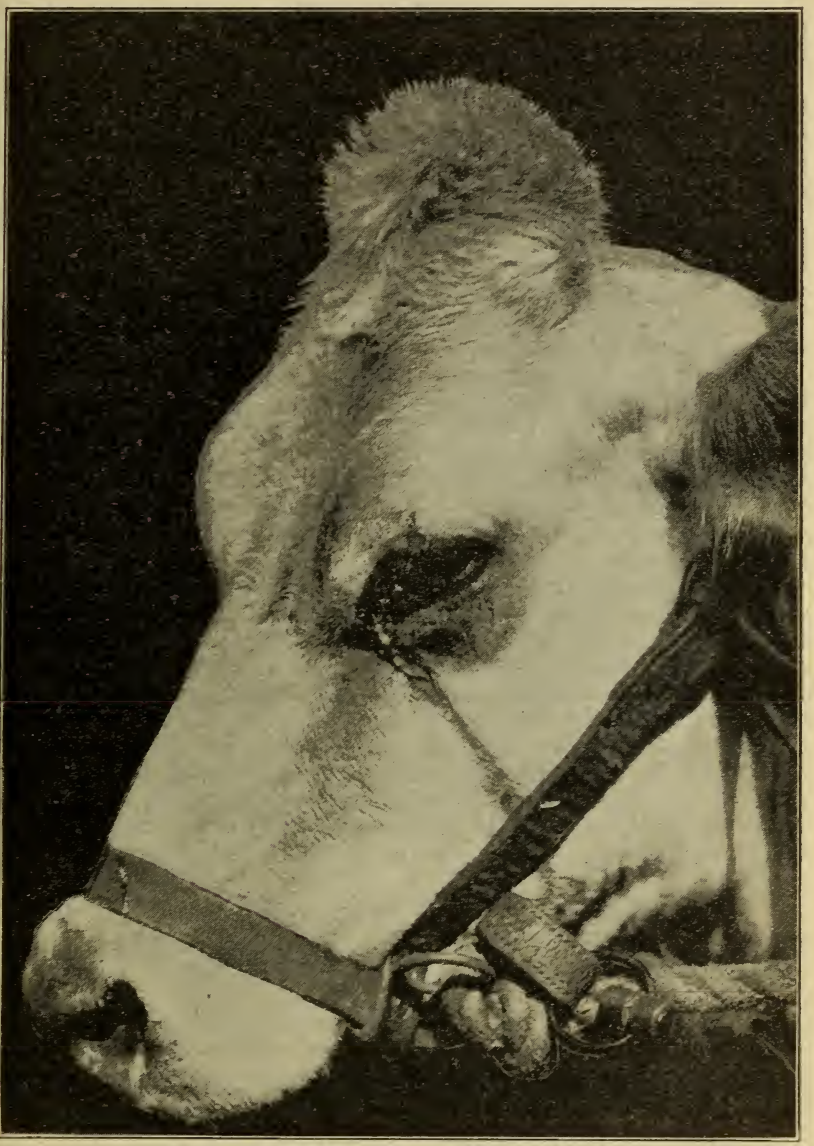

FIG. 8.—Cow showing typical ophthalmic reaction. The discharge from the eye may be more abundant or somewhat less than indicated in the picture

6. Take observations following the placing of the sensitizing disk, if possible, commencing at about the second hour and repeating them every two hours for not less than eight hours. These observations are desirable, though not essential.

7. If the test is made in conjunction with the intradermic method, the diagnostic disks are placed, preferably, on or about the seventy-second hour following the sensitizing disk. If it is made with the subcutaneous test, it is permissible to place the diagnostic disk at about the fourteenth hour after the sensitizing disk or at the time of the second postinjection temperature.

8. Observations, following the placing of the diagnostic disks, should be taken at the second hour and at not to exceed two-hour intervals up to the eighth hour or longer. More frequent observations may be taken of badly infected herds; some animals are very slow in responding to the test.

9. The proper diagnostic dose of tuberculin is contained in one of the specially prepared disks of the Bureau of Animal Industry. Occasionally it has been found advisable to use a second disk if it appears that there is a tendency of the reaction to be retarded or not well defined. An additional tablet may be given at any time after the first diagnostic disk has been used.

10. Reactions are indicated usually by lachrymation and redness of the conjunctiva, possibly swelling, and a free discharge of mucopurulent pus. This typical discharge should not be confused with an occasional mucous discharge sometimes seen at the inner canthus as a result of the introduction of any foreign material. Occasionally a tuberculin disk may cause this slight, faint, mucous discharge of whitish appearance, especially if the tuberculin disks have not been properly placed.

11. The observations made following the use of ophthalmic tuberculin should be reported on prescribed forms and in accordance with the following symbols: *A.* Animals negative to the test should be reported "N" (negative). *B.* The animals whose tested eye exhibits dark, albuminous exudate equivalent in volume to 8 minims or more should be reported by the use of the symbol "P X." If the exudate exhibits granular casts or flakes of a yellowish tint, the report should read "P X X." When the exudate tends to smear over the ball and pupil of the eye, note the fact with the symbol "P X X X." The following table indicates the proper method of reporting ophthalmic observations:

Animal No.	2 p. m.	4 p. m.	6 p. m.	8 p. m.	Remarks
1	N	MV	PX	PXXX	Reacted.
2	N	N	N	N	Negative.

12. Animals indicating less than 8 minims of a typical discharge should be classed as suspicious and the amount of discharge shown by the letter "M" followed by the Roman numeral. If the amount of discharge is, for example, 4 minims, indicate by "MIV."

13. In classing animals as suspects extreme care should be used to take into consideration the characteristic appearance of the discharge and not to class merely as suspicious those animals which give only a limited amount of very typical discharge. Such animals should be classed as reactors.

COMBINATION TUBERCULIN TESTS

By a combination tuberculin test is meant the grouping of two or more known methods of testing. In applying combination tests it is strongly emphasized that the successful application of two or more simultaneously depends entirely upon the attention given to each. The usual procedure is to use only two methods. It is at times advisable, however, to apply all three of the major tests. A combination deemed most advisable by State and bureau officials is now necessary as the final test on all herds in process of becoming accredited.

Since the inauguration of the cooperative tuberculosis-eradication work, May 1, 1917, much time has been spent in demonstrating the value of combining methods; 5,783,179 tests were made between October 1, 1920, and June 30, 1927, using the various combinations. It was recognized for many years that there were conditions in certain herds which necessitated the employment of the most stringent test known if the disease was to be controlled and eventually eradicated. In those particular herds it was found that certain animals failed to react to one test and gave a violent reaction to another one applied simultaneously. Herds of this character should have check tests applied. Without the previous history of the herd, it is not always possible to determine when such combinations should be used. It may be accepted, however, as a general principle that in localities where the percentage of infection is known to be large it is advisable to apply check tests when the herd is first tested. It is also advisable to apply combination tests to all animals which are to be added to herds whose histories are known to be good and to those animals which have been previously classified as suspicious.

Combinations of tests are a relatively new procedure. Therefore it is necessary that close study be given to the application of the various combinations if they are to be of value. The following are the combinations which it is possible to use:

Intradermic-ophthalmic-subcutaneous combination.
Subcutaneous-ophthalmic combination.
Intradermic-ophthalmic combination.
Intradermic-subcutaneous combination.

THE INTRADERMIC-OPHTHALMIC-SUBCUTANEOUS COMBINATION

The intradermic-ophthalmic-subcutaneous combination is undoubtedly the most searching combination which can be used. It has assumed a prominent place in the testing work in a number of States. It is found in this grouping that an occasional reactor results from one of the tests which is not detected by either of the other two, and this is especially true in some badly infected herds. One point in connection with this combination refers to the use of the subcutaneous test. This should be used at the completion of the intradermic and ophthalmic methods. Only such animals as have failed to give a typical reaction to the other two methods should be injected with subcutaneous tuberculin. This is because it has been shown in many instances that certain reactors to other tests used in combination do not react again when the subcutaneous test has been used as a check. Therefore, to avoid confusing the operator,

and possibly the owner, as to the accuracy of tuberculin, its use is not advised upon known reactors. This should be remembered when the three tests are applied in combination, as most cattle owners are not likely to understand why the combination should not be completed on all cattle under test. The technic of the intradermic-ophthalmic-subcutaneous combination test is as follows:

DIRECTIONS FOR APPLYING

1. Prepare the herd for test in accordance with directions for a single tuberculin test.
2. All necessary instruments should be available and thoroughly clean. Sufficient quantities of tuberculin should be provided.
3. Inject the intradermic tuberculin, sensitizing the eye for the ophthalmic test at the same time by the use of one disk.
4. Make an observation for intradermic results at or about the seventy-second hour. Place the diagnostic ophthalmic disks at about the same time.
5. Begin preliminary temperatures for the subcutaneous test following the placing of the diagnostic ophthalmic disks.
6. Take observations on the ophthalmic test for a period of eight hours or longer.
7. In accordance with directions for individual tests, take observations on ophthalmic and intradermic tests, classifying all animals as "reactors," "suspects," or "passed."
8. At the conclusion of classification of animals inject with subcutaneous tuberculin only those animals which failed to give positive reactions to either the intradermic or ophthalmic test. At least twice the minimum dose of subcutaneous tuberculin prescribed should be administered.
9. Complete the tuberculin test, observing the eye frequently during the period covered by the taking of temperatures.
10. Make a final record of all tests.

THE SUBCUTANEOUS-OPHTHALMIC COMBINATION

The combination of the subcutaneous and ophthalmic tests is one very much used, and it has been found to give very satisfactory results. It is recommended that this combination be alternated with the intradermic-ophthalmic test wherever continuing infection is found in herds. It does not afford opportunity for the testing of so large a number of herds as can be accomplished by the intradermic-ophthalmic method. In connection with its use the question of the length of time regarded necessary to produce sensitization of the eye for the ophthalmic disks is important. When used with the subcutaneous test it is not practicable to have more than 16 hours of sensitization. However, even this period has shown wonderfully good checks against the subcutaneous even though the discharge may not be quite so copious. It has been found that in some cases the injection of the subcutaneous tuberculin probably aids, in a marked manner, the ophthalmic reactions. The technic of this combination is as follows:

DIRECTIONS FOR APPLYING

1. Prepare the herd for test in accordance with suggestions for the subcutaneous test alone.
2. Sensitize the eye for the ophthalmic test, after which begin preliminary temperatures for the subcutaneous test.
3. The subcutaneous test is conducted in accordance with directions.
4. Place the diagnostic disks at about the tenth hour after the injection of subcutaneous tuberculin or about the second postinjection reading.
5. Make observations at the same time as temperature readings, or more frequently in questionable cases.

FIG. 9.—Part of a herd of 76 head of cattle, 57 of which were found to be reactors as a result of the intradermic-ophthalmic combination test. All the cattle in this picture were diseased, as was proved by the autopsy conducted on them

6. The ophthalmic test is completed at about the same time as the subcutaneous.
7. Report observations in accordance with the prescribed code.

THE INTRADERMIC-OPHTHALMIC COMBINATION

For purposes of economy and practicability the combination of the intradermic and ophthalmic tests, if properly applied, is more desirable than any other. Information at hand indicates that these two tests afford an excellent check against each other, and that the results following their use have been very good. Figures 9 and 10 show a herd of cattle tested by this combination and a single animal from the herd which also reacted to both tests. This combination is especially valuable in the handling of an area problem

where many badly infected herds may be encountered. If properly understood it should be extremely valuable to the busy practicing veterinarian who, by reason of a large practice, is almost prohibited from using the other combinations of tests. If the herd history indicates a possibility of infection, the eye may be sensitized at the time the test is begun, and thus a complete check effected during the period ordinarily used in making one test.

It has been shown as a result of experience that this combination especially pleases cattle owners, because they are not required to restrain their cattle for a long time.

FIG. 10.—This animal, which reacted to both the intradermic and ophthalmic tuberculin tests, was 18½ years old and part of the herd shown in Figure 9. This cow was found to be a generalized case on autopsy. Undoubtedly the animal was a prolific source of the very heavy infection found in the herd

DIRECTIONS FOR APPLYING

1. The herd is prepared for the test in accordance with directions for other tests.

2. Make the intradermic injections and place the sensitizing ophthalmic disks at the same time.

3. Place the diagnostic ophthalmic disks at or about the seventy-second hour.

4. Take ophthalmic observations, commencing the second hour after the instillation of the diagnostic disks and at least every two hours thereafter for a period of eight hours, or longer, if necessary. More frequent observations may be made in infected herds.

5. At about the eighth hour after placing the diagnostic disks the final observations are made for the combined test.

6. It is occasionally advisable to make intradermic observations at a period later than the seventy-second hour on those animals which have not reacted to either the intradermic or ophthalmic test at the seventy-second hour.

7. Additional instillation of ophthalmic disks may be made during the course of the test in doubtful cases.

8. Records are completed in accordance with the prescribed codes and forms in use.

THE INTRADERMIC-SUBCUTANEOUS COMBINATION

Combining the subcutaneous and intradermic methods of testing affords no special features, and is not recommended. It involves a considerable amount of time in application, and if these two tests are to be used the ophthalmic should also be used, which would place it in the triple combination class. If at any time it is used the intradermic tests should be completed prior to the injection by the subcutaneous method, and the reports made in accordance with instructions governing each test.

TUBERCULIN TESTING OF SWINE AND FOWLS

The question of tuberculosis in swine and poultry in certain of the hog-raising States of the Middle West is a very important one.

It is known that swine become infected through the ingestion of germ-laden feed from cattle sources, such as unpasteurized milk from creameries; the following of feeder cattle in feeding lots; and the feeding of tuberculosis carcasses or slaughterhouse offal. It is also believed that a certain proportion of diseased swine contract tuberculosis by eating uncooked garbage. The rapid growth of the area plan of eradication has also demonstrated that a considerable percentage of infection in swine can be traced to tuberculous poultry.

It will be readily seen that the sources of infection are sufficiently constant to maintain the already very high percentage of tuberculosis known to exist in swine. It is therefore deemed advisable to test all breeding animals maintained on premises where tuberculosis is known to exist or where such swine have access to possible infected material.

In the event that tuberculosis is found in the swine, the entire herd had better be disposed of for slaughter unless those that have passed the test are valuable for breeding purposes.

The best method of testing swine is the intradermic method, the injection being made into the skin near the base of one of the ears of the animal. Observations should be made in accordance with the instructions for the test as it pertains to cattle. Removal of reactors and cleaning and disinfecting should be practiced if the tuberculin testing is to be a success.

In connection with the tuberculin testing of fowls full information is available and is contained in Farmers' Bulletin 1200, United States Department of Agriculteure, "Tuberculosis of Fowls."

IDENTIFICATION OF TEST ANIMALS

The identification of all cattle tested is very important in the control and eradication of tuberculosis. It affords a protection to the

practitioner who may test cattle for occasional unscrupulous dealers, who possibly would be inclined to shift untested cattle into lots supposedly passed. Likewise this practice of identifying all animals, both reactors and passed, by means of ear tags and a brand, if diseased, facilitates their interstate movement and the payment of indemnity, as the Federal regulations governing such shipments and indemnity claims require proper identification of passed animals and the branding of tuberculous ones.

All grade animals which have successfully passed a tuberculin test should be identified by a metal tag approved by the Bureau of Animal Industry or the State livestock sanitary officer of the State in which the cattle are tested. It should be of a permanent type and not easily detached, and placed in right ear.

Purebred animals which have successfully passed the test need not be tagged except on the request of the owner. This class of animals, however, is readily identified on the test records by the registration numbers, names, and descriptions.

All reacting animals should be handled in accordance with the regulations of the various States. They should be branded and tagged in order that their identity may be permanently established.

All reacting animals which are to be moved interstate must be marked for identification by attaching to the left ear a metal tag bearing a serial number and a proper inscription of the State or Federal Government showing the animal to be diseased. Such diseased cattle must also be permanently identified by branding on the left jaw the letter **T**. This regulation likewise applies to animals for which claim for Federal indemnity is to be made.

Animals classified as "suspicious" should also be identified by proper tags.

POST-MORTEM EXAMINATION OF REACTING CATTLE

It is important that the disease be disclosed in animals which have reacted to tuberculin. The predominant idea of endeavoring to show a reasonable percentage of visible lesions is based upon the knowledge that cattle owners for whom the work is done are much better satisfied to know that infection was found after the animals had been condemned. The writers do not emphasize the desirability of disclosing lesions for the purpose of basing the accuracy of the tuberculin test upon the autopsy findings. It is fully appreciated that a certain percentage of animals, with lesions of a very obscure nature that would be overlooked in ordinary routine examination, will react to tuberculin.

Nevertheless every veterinarian should familiarize himself with the technic of conducting a post-mortem examination. A vast majority of the members of the profession probably know the primary seats of tuberculous infection. There is the possibility, however, of tuberculous foci being found in obscure or isolated organs or glands. Much work has been done during the last two years in investigating so-called "no-lesion" cases in tuberculin reactors. It was found that a much larger number of skin lesions were present than had been supposed. For instance, it was found that during the period from January 1 to March 31, 1922, approximately 2½ per cent of the 20,683 reacting cattle slaughtered under official inspection showed

skin lesions only. It is further known that many cases of early infection will react in which it will be hard to disclose lesions even of microscopic size. Further, it should be remembered that a lesion microscopic in character will produce a reaction and that it would be impossible to disclose foci of this size in the carcass of an average-size animal. Records compiled by the bureau show that over 50 per cent of so-called no-lesion cases are from herds in which infection is known to exist.

An interesting publication, Circular No. 32, The Regional Lymph Glands of Food Animals, may be obtained on application to the department.

NECESSARY WORK FOLLOWING TUBERCULIN TESTING

One of the most important phases of tuberculosis-eradication work is the attention given to herds after the tuberculin test has been completed, especially those in which infection is found. Histories of herds in which infection continues to exist or in which infection recurs too often points to, and is the result of, failure to adopt the necessary sanitary measures or to guard against the entrance of infected cattle. No test should be regarded as completed until the owner has been fully advised of all the possible agencies which might at times prevent the establishment and maintenance of tuberculosis-free herds. Following are the essential safeguards:

All reactors should be identified and immediately removed from the herd or properly segregated until such time as they can be disposed of in accordance with the State regulations.

The premises occupied by diseased cattle should be immediately cleaned and disinfected under proper supervision. Farmers' Bulletin 954 of the United States Department of Agriculture, The Disinfection of Stables, prescribes thorough methods of accomplishing this work.

No cattle should be added to the herd without having satisfactorily passed a tuberculin test.

The cattle of the herd should not be permitted to associate with other cattle except those that have passed a satisfactory tuberculin test.

Building alterations should be made to permit the housing of the herd under proper sanitary precautions.

Special attention should be given to watering troughs, some types of which, even individual receptacles, have been found to be prolific spreaders of tuberculosis. Ponds to which diseased cattle have access are sources of danger.

The feeding of calves and swine with unpasteurized products from herds containing diseased cattle or from creameries should be guarded against. The question of tuberculosis in either swine or poultry should be thoroughly looked into and necessary steps taken to guard the herd or flock.

A herd from which the reactors are removed should be retested within a period of from 90 days to 6 months by proper combinations of tests.

The advantage to be derived from advising the owner along the lines indicated are clearly shown by the history of numerous herds

which have been freed of disease and which have been maintained under sanitary conditions. Figure 11 shows the interior of a type of barn which, while not practicable for the average farmer, is considered as a model. When maintained under conditions like these after being established as free of disease the herd should always normally remain in that condition.

CONCLUSIONS

The proper understanding of the nature of tuberculosis coupled with the use of properly prepared tuberculin by a veterinarian skilled in its application will lead to most satisfactory results in testing.

To obtain the best results the different methods of tuberculin testing and their combinations must be thoroughly studied.

FIG. 11.—Interior of a model sanitary dairy barn. Note cleanliness of stable and cows

Following the application of the test, the identification of the animals and careful post-mortem work on reactors are essential.

Veterinarians should be prepared to conduct the work in such manner as to impress livestock owners with its value and the importance of selecting healthy foundation stock. Inspiring confidence in the herd owner leads to a willing compliance with instructions and suggestions.

Finally the cleaning and disinfection of the premises and the necessary precautions to guard against reinfection of the herd must be carried out.

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